Plant Signaling & Behavior 7:8, 957-960; August 2012; © 2012 Landes Bioscience

The use of light in prey capture by the tropical pitcher plant *Nepenthes aristolochioides*

Jonathan A. Moran,[1,*] Charles Clarke[2] and Brent E. Gowen[3]

[1]School of Environment and Sustainability; Royal Roads University; Victoria, BC Canada; [2]School of Science; Monash University Sunway Campus; Selangor, Malaysia; [3]Department of Biology; University of Victoria; Victoria, BC Canada

Keywords: drosophila, nepenthes, pitcher plant, prey attraction, visual signaling

Nepenthes pitcher plants deploy tube-shaped pitchers to catch invertebrate prey; those of *Nepenthes aristolochioides* possess an unusual translucent dome. The hypothesis was tested that *N. aristolochioides* pitchers operate as light traps, by quantifying prey capture under three shade treatments. Flies are red-blind, with visual sensitivity maxima in the UV, blue and green wavebands. Red celluloid filters were used to reduce the transmission of these wavebands into the interior of the pitchers. Those that were shaded at the rear showed a 3-fold reduction in Drosophila caught, relative to either unshaded control pitchers, or pitchers that were shaded at the front. Thus, light transmitted through the translucent dome is a fundamental component of *N. aristolochioides'* trapping mechanism.

Carnivorous pitcher plants of the genus Nepenthes attract arthropod prey using a combination of color patterns, scent and nectar.[1,2] Typical Nepenthes pitchers are tubular/ovoid in shape, with an opening at the top, surrounded by the peristome (the collar-shaped structure surrounding the pitcher mouth), and a lid situated above. Prey fall into these pitchers under gravity. Capture mechanisms include a wettable peristome,[3,4] slippery wax crystals on the inner pitcher wall,[5-7] and viscoelastic fluid.[2,8-10]

Nepenthes aristolochioides (Jebb and Cheek) is a Sumatran montane species with unusual pitcher morphology:[11,12] the rear upper portion of the *N. aristolochioides* pitcher is expanded into a pronounced dome, with the mouth sitting at the front, rather than the top (**Fig. 1A**). The dome is translucent (**Fig. 1B**), and it has been proposed that it serves to draw and retain prey that are attracted to light.[12] Field observations show an apparent specialization in capturing small Diptera and it has been postulated that the translucent dome of *N. aristolochioides* pitchers plays a key role in the selective capture of these insects.[11,12] The aims of the study were to test the hypothesis that *N. aristolochioides* pitchers function as light traps for small Diptera, and to investigate morphological characteristics that might facilitate this strategy.

Pitcher Morphology

The domed structure of the pitcher is demonstrated in **Figure 1A**. At left is the pitcher interior; at right is the exterior. Areas denoted by letters *c* to *f* are shown in detail in the lower panels. The translucence of the dome is shown in **Figure 1B** (the overhanging pitcher lid was removed to allow the interior to be photographed). **Figure 1C** shows a section through the rear, domed wall. Note digestive gland on interior wall and trichomes on the exterior. The typical anisotropic microstructure of the peristome is shown in **Figure 1D**. A section through the front wall of the pitcher is presented in **Figure 1E**. This is *ca.* 30% thicker than that of the dome (**Fig. 1C**). The digestive glands of the rear pitcher wall are shown in **Figure 1F**. Epicuticular waxes are absent.

Color Pattern

The percentage of white (translucent) area at the rear of the pitcher is almost twice that at the front (mean, SE = 65.7 ± 4.3% vs. 34.3 ± 4.3%, respectively. $t = -8.727$, $p < 0.0001$).

Prey Capture

Pitchers that were shaded at the rear (i.e., reduced green, blue and UV light transmitted through the dome) caught significantly fewer Drosophila (21.7 ± 7.0%, n = 6) than either the control pitchers (68.3 ± 9.4%, n = 6), or those shaded at the front (56.7 ± 11.4%, n = 6; $F = 5.74$, Type III SS between treatments = 1.059, Type III SS residual = 1.384, $p = 0.014$). There was no significant difference between the latter two treatments (**Fig. 3**).

In the current study, no flies were observed slipping on the peristomes of *N. aristolochioides* pitchers, despite their being saturated with nectar. The peristome microstructure is identical to that of typical Nepenthes, (**Fig. 1D**) so loss of functionality is due to its unusual orientation away from the vertical (**Fig. 1A**); it provides a stable foothold even when wet. In lieu of the usual combination of slippery peristome and gravity, the prey is persuaded to convey itself into the trap under muscle power. In a typical interaction, a fly alights on the *N. aristolochioides* pitcher to feed at the

*Correspondence to: Jonathan Moran; Email: jonathan.moran@royalroads.ca
Submitted: 04/24/12; Revised: 05/28/12; Accepted: 05/29/12
http://dx.doi.org/10.4161/psb.20912

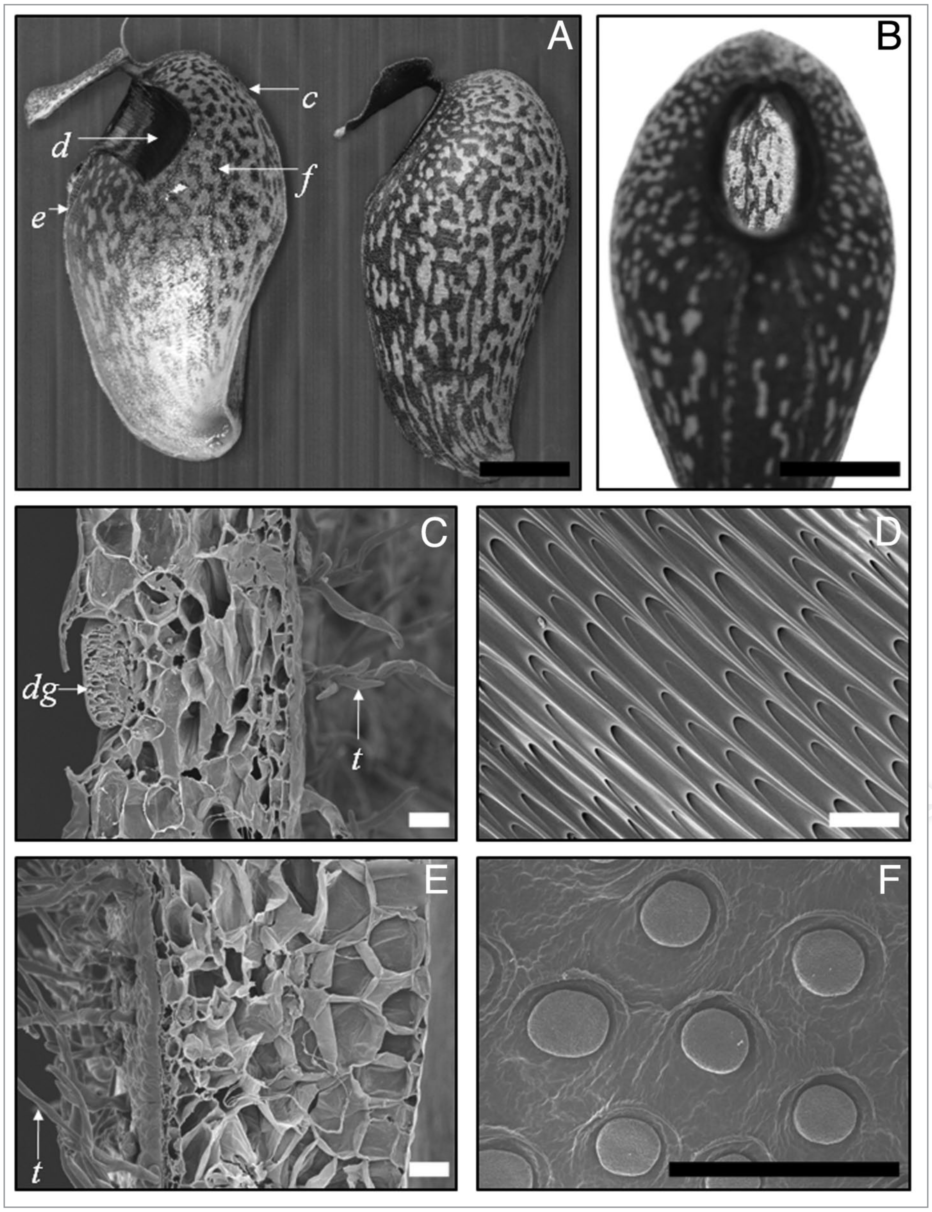

Figure 1. *Nepenthes aristolochioides* pitcher morphology. (A) Interior (left) and exterior (right) views. Arrowed areas *c* to f are shown in detail in correspondingly-lettered lower panels. (B) Frontal view of pitcher showing translucence of the domed rear wall. Lid removed to allow photography. (C) Scanning electron micrograph (SEM) of rear (domed) section of pitcher wall. *DG* = digestive gland; *T* = trichome. (D) SEM of peristome surface, showing typical anisotropic arrangement of epidermal cells. (E) SEM of front section of pitcher wall. *T* = trichome. (F) Digestive glands on interior wall. Epicuticular waxes are absent. Scale bars: black = 1 cm; white = 50 μm.

small extrafloral nectaries on the exterior. Trichomes on the exterior wall provide a secure foothold (**Fig. 1C and E**). Eventually it finds the peristome, the site of the major nectaries and greatest nectar volume. Once on the peristome, its view to the outside is occluded by the overhanging lid, the underside of which is a dark red/brown color (**Fig. 1A**): in a reversal of the usual situation in Nepenthes pitchers, the exit is effectively dark. In contrast, the translucent dome at the rear of the pitcher is a zone of brightness typical of an exit (**Fig. 1B**), toward which the fly proceeds, eventually leaving the peristome and entering the pitcher body. The inner walls produce copious amounts of viscoelastic fluid, which retains the fly, conveying it to the pitcher base, where it is digested.

Several morphological/anatomical features contribute to the effectiveness of this light trap. First, the pitcher lid is oriented to cut off light from the exit. Second, the evolutionary loss of a wax zone on the inner pitcher wall (**Fig. 1F**) reduces interception of transmitted light. Third, the thinness of the pitcher wall in the rear, domed section (ca. 30% thinner than the front wall; **Fig. 1C and E**), combined with the lack of red pigmentation in this area, allow transmission of light into the pitcher interior (**Fig. 1B**), providing a false (i.e., reversed) orientation cue that leads prey further into the trap, rather than to the exit. The results of the shade experiment demonstrate that shading of this domed area significantly reduces prey capture by a factor of three (**Fig. 3**), supporting the hypothesis that light transmission is a key component of the carnivorous syndrome in this species. It is important to note that the red celluloid filters did not physically impede access to the pitchers. In most cases, the flies remained on the pitchers during the entire seven-hour period, either feeding on the nectaries on the outside of the pitcher, or resting. They did not fly straight to the peristome, even in the control treatment. Rather, they typically walked over the surface of the pitcher body. The only behavioral difference between the various treatments was that, in the rear shade treatment, the flies did not often proceed from the peristome into the pitcher body. Given that the opening of the *N. aristolochioides* pitcher is at the front, then if the filter impeded access to the opening, it would be expected that the front shade treatment would show the lowest rate of capture. This was not the case, as in this treatment the pitchers caught approximately three times more flies than pitchers that were shaded at the rear. Further, there was no significant difference between the numbers of flies caught in the front-shaded treatment, and the control pitchers that were completely unshaded.

The unusual pitcher morphology of *N. aristolochioides* is shared with one congener, *Nepenthes klossii* (Ridl.).[12] It is also similar to that of three New World pitcher plants (Sarraceniaceae), *Darlingtonia californica* (Torr.), *Sarracenia minor* (Walt.) and

Sarracenia psittacina (Michx.).[18] To date, studies comparable to ours have not been performed on these species. If it were demonstrated that these species use transmitted light in a similar way to *N. aristolochioides*, it would provide a powerful and novel example of convergent evolution encompassing two Families and three Genera.

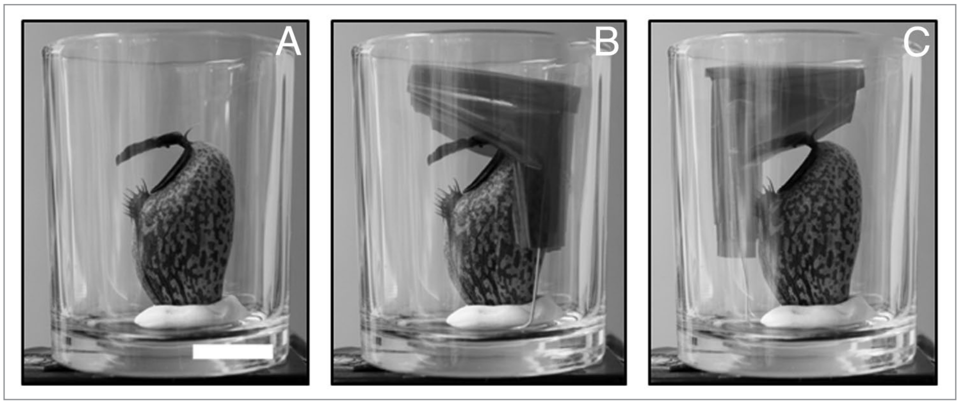

Figure 2. *Nepenthes aristolochioides* prey capture experimental treatments. (A) Control. (B) Red celluloid shade at pitcher rear. (C) Red shade at pitcher front. Scale bar = 2 cm.

Materials and Methods

Six tissue-cultured *N. aristolochioides* plants were obtained commercially (Hawaiian Botanicals, Richmond, BC Canada). Only fully-opened, mature pitchers were used. Tissues were double fixed in glutaraldehyde and OsO_4, dehydrated through a graded ethanol series, critical point dried, mounted on stubs, and sputter coated with gold. Images were obtained on a Hitachi S-3500N scanning electron microscope (Hitachi Inc., Toronto, Canada) at 15 kV.

N. aristolochioides pitchers have the reticulate patterning typical of many Nepenthes. To test whether the rear (domed) region of the pitcher possessed a greater proportion of translucent, white area than the front, digital images were taken using a Canon 40D camera (Canon Inc., Tokyo, Japan), then analyzed using ImageJ v.1.44p (http://rsbweb.nih.gov/ij/index.html). For each pitcher (n = 13), a lateral image was divided vertically into equal-area front and rear portions, in each of which the number of pixels representing pigmented (red/brown) or translucent (white) areas, were counted using the Freehand Selection tool and Measure function. These values were converted to % translucent area, and square-root arcsine transformed.[13] A paired t-test was performed to compare % translucent area between the front and rear portions of the pitchers.

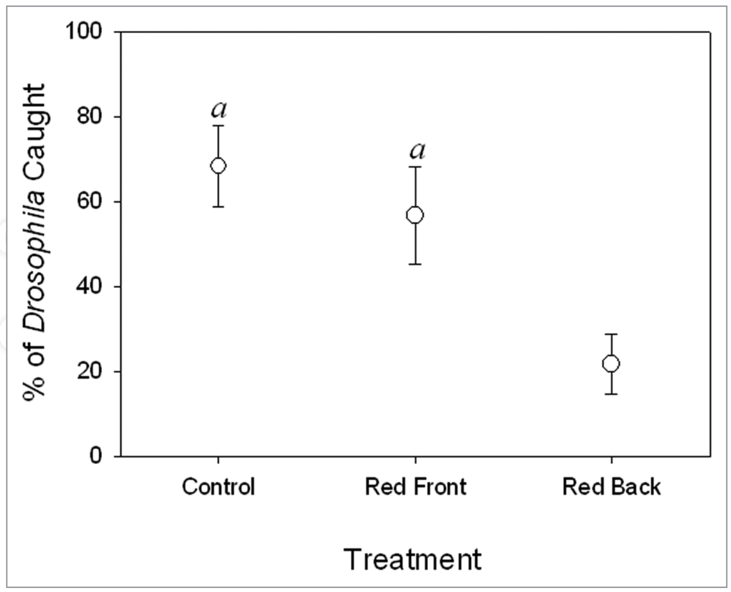

Figure 3. The effect of pitcher shading on % capture of fruit flies (*Drosophila melanogaster*) by *Nepenthes aristolochioides*. Values are means ± 1 SE.; n = 6 pitchers per treatment. Treatments that do not differ significantly (p > 0.05, Tukey test) share the same italicized letter.

For the prey capture experiment, three pitchers were removed from each of the six plants (n = 18) and placed in clear glass cups, held upright using non-toxic, volatile-free putty. The pitchers were removed from the plants by cutting the tendril; thus, the pitchers remained intact and did not lose any of their viscoelastic fluid. Each pitcher was then randomly allocated one of three treatments: (1) Control (**Fig. 2A**); (2) Red celluloid film held by a thin wire frame over the rear of the pitcher (**Fig. 2B**). This reduced the amount of light entering the pitcher through the translucent dome. Flies are trichromats, with sensitivity maxima in the UV, blue and green wavebands, i.e., they are red-blind.[14-17] Spectral analysis of the red celluloid film, using a spectroradiometer (USB4000, Ocean Optics Inc., Dunedin, FL), demonstrated that it blocked ca. 50, 70 and 80% of incident radiation (indirect natural sunlight) in the UV, blue and green wavebands, respectively; and (3) Red celluloid film positioned over the front of the pitcher (**Fig. 2C**). This treatment did not reduce light entering the pitcher via the translucent dome. It was used to control for possible chemical effects (e.g., volatiles) of the shade apparatus, as well as providing the same overall level of shade as treatment 2. To each glass cup were added 10 non-anaesthetized wild-type *Drosophila melanogaster*. Each glass was sealed with colorless celluloid film, perforated with air holes. All pitchers were left in indirect natural light for 7 h, after which the number of flies caught was counted. Since the plants were clonal (i.e., possibly genetically identical, having been tissue cultured from the same original stock), we erred on the side of caution and did not treat each plant as a statistically independent unit— to have done so would have constituted pseudoreplication. We instead accepted this lack of genetic variability and treated all pitchers in the study as deriving from a genetically homogeneous "pool," from which we selected pitchers at random. At six fortnightly intervals, three mature pitchers were selected and each randomly allocated to one of the three treatments, such that all treatments were represented at each interval. A repeated measures analysis of variance (ANOVA) showed no effect of sampling date on prey capture. As a result, we re-analyzed the data using a simple one-factor ANOVA. Prior to analysis, % prey capture data were square-root arcsine transformed.[13] Levene's and Kolmogorov-Smirnov/Lilliefors tests confirmed homoscedasticity and normality, respectively. Following ANOVA, we performed a Tukey HSD all-pairwise comparison of means. SigmaPlot v.12 (Systat Software Inc., San Jose) was used for all statistical tests.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgements

We thank Alison Moran for valuable discussions, Royal Roads University for funding the study, and Vincent Bonhomme and an anonymous reviewer for improving the manuscript considerably.

References

1. Moran JA, Booth WE, Charles JK. Aspects of pitcher morphology and spectral characteristics of six Bornean Nepenthes pitcher plant species: Implications for prey capture. Ann Bot (Lond) 1999; 83:521-8; http://dx.doi.org/10.1006/anbo.1999.0857.
2. Giusto BD, Grosbois V, Fargeas E, Marshall DJ, Gaume L. Contribution of pitcher fragrance and fluid viscosity to high prey diversity in a Nepenthes carnivorous plant from Borneo. J Biosci 2008; 33:121-36; PMID:18376077; http://dx.doi.org/10.1007/s12038-008-0028-5.
3. Bohn HF, Federle W. Insect aquaplaning: Nepenthes pitcher plants capture prey with the peristome, a fully wettable water-lubricated anisotropic surface. Proc Natl Acad Sci USA 2004; 101:14138-43; PMID:15383667; http://dx.doi.org/10.1073/pnas.0405885101.
4. Bauer U, Bohn HF, Federle W. Harmless nectar source or deadly trap: Nepenthes pitchers are activated by rain, condensation and nectar. Proc Biol Sci 2008; 275:259-65; PMID:18048280; http://dx.doi.org/10.1098/rspb.2007.1402.
5. Juniper BE, Burras J. How pitcher plants trap insects. New Sci 1962; 13:75-7.
6. Riedel M, Eichner A, Meimberg H, Jetter R. Chemical composition of epicuticular wax crystals on the slippery zone in pitchers of five Nepenthes species and hybrids. Planta 2007; 225:1517-34; PMID:17109149; http://dx.doi.org/10.1007/s00425-006-0437-3.
7. Gaume L, Di Giusto B. Adaptive significance and ontogenetic variability of the waxy zone in *Nepenthes rafflesiana*. Ann Bot 2009; 104:1281-91; PMID:19805403; http://dx.doi.org/10.1093/aob/mcp238.
8. Gaume L, Forterre Y. A viscoelastic deadly fluid in carnivorous pitcher plants. PLoS One 2007; 2:1185; PMID:18030325; http://dx.doi.org/10.1371/journal.pone.0001185.
9. Bauer U, Clemente CJ, Renner T, Federle W. Form follows function: morphological diversification and alternative trapping strategies in carnivorous Nepenthes pitcher plants. J Evol Biol 2012; 25:90-102; PMID:22023155; http://dx.doi.org/10.1111/j.1420-9101.2011.02406.x.
10. Bonhomme V, Pelloux-Prayer H, Jousselin E, Forterre Y, Labat JJ, Gaume L. Slippery or sticky? Functional diversity in the trapping strategy of Nepenthes carnivorous plants. New Phytol 2011; 191:545-54; PMID:21434933; http://dx.doi.org/10.1111/j.1469-8137.2011.03696.x.
11. Clarke CM. Nepenthes of Sumatra and Peninsular Malaysia. Kota Kinabalu, Malaysia: Natural History Publications 2001.
12. Nerz J. Rediscovery of an outstanding Nepenthes: *N. aristolochioides* (Nepenthaceae). Carn Plant Newslett 1998; 27:101-14.
13. Sokal RR, Rohlf FJ. Biometry. New York: WH Freeman and Co. 1981.
14. Briscoe AD, Chittka L. The evolution of color vision in insects. Annu Rev Entomol 2001; 46:471-510; PMID:11112177; http://dx.doi.org/10.1146/annurev.ento.46.1.471.
15. Stavenga DG. Angular and spectral sensitivity of fly photoreceptors. II. Dependence on facet lens F-number and rhabdomere type in Drosophila. J Comp Physiol A NeuroetholSens Neural Behav Physiol 2003; 189:189-202; PMID:12664095.
16. Stavenga DG. Visual acuity of fly photoreceptors in natural conditions-dependence on UV sensitizing pigment and light-controlling pupil. J Exp Biol 2004; 207:1703-13; PMID:15073203; http://dx.doi.org/10.1242/jeb.00949.
17. Yamaguchi S, Desplan C, Heisenberg M. Contribution of photoreceptor subtypes to spectral wavelength preference in Drosophila. Proc Natl Acad Sci USA 2010; 107:5634-9; PMID:20212139; http://dx.doi.org/10.1073/pnas.0809398107.
18. Juniper BE, Robins RJ, Joel D. The carnivorous plants. London, UK: Academic Press 1989.